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A PRELIMINARY SURVEY OF DIATOM TAXA FROM OLD-GROWTH FOREST TIP-UP POOLS IN A SOUTHEASTERN INDIANA FLATWOODS

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ABSTRACT. The forests of the Illinoian tillplain in southeastern Indiana are characterized by unique hydrology, soil features, and woody species composition. These hydro-mesophytic forests are typified by their poor drainage and lack of topography. Topographic variation is limited to pit-and-mound topography resulting from tree falls. Tip-up pools formed from tree-fall events represent a unique microhabitat in the forest matrix. Although much attention has been given to the woody species composition of these forests, less is known about the biota within these pits. In June 2009, nine tip-up pools from Tribbetts Woods, an old-growth remnant forest in Jennings County, Indiana, USA were sampled to determine their diatom composition. A total of 80 diatom taxa were identified from the pool sediments and leaf litter. Sediment samples contained 39 diatom taxa while 71 were found in the leaf litter samples.

Keywords: diatoms, Indiana, temporary pool, tip-up, Tribbetts Woods

INTRODUCTION

It has been well established that temporary pools are critical components within the landscape of terrestrial communities, and offer unique environmental conditions, vital areas for reproduction, and potential points of refugia (De Meester et al. 2005; Boven et al. 2008). The unique natural histories of temporary pool inhabitants can greatly enhance the beta-diversity of a region (Oertli et al. 2002; Colburn 2004; Williams et al. 2004; De Meester et al. 2005).

Studies of these temporary systems often bypassed cryptic groups of organisms in favor of macroscopic taxa (e.g., macroinvertebrates, amphibians, vascular plants) (Colburn 2004). Of these cryptic microbial assemblages, algae are of particular interest since they can contribute greatly to the primary production of these habitats (Sands 1984). Diatoms are especially well suited to inhabit temporary aquatic habitats due to their ubiquity and cosmopolitan distributions. Their ecological success, in part, stems from their extensive diversity and ability to colonize and thrive in a myriad of freshwater, marine, wetland, and even some terrestrial (i.e., damp cliff walls) habitats. Despite their extensive distribution there are many aquatic habitats, with suitable light requirements, in which diatoms have not been well inspected, such as temporary or vernal pools. The objective of this study was to create a baseline inventory of diatom species inhabiting unique temporary tip-up pools from an old growth forest remnant in the Illinoian tillplain of southeastern Indiana.

Site description.—Tribbetts Woods is a 13.4 hectare old-growth forest remnant located in Jennings County, Indiana (Jackson & Barnes 1975). The woods is located in the Illinoian till plain of southeastern Indiana and falls in the area of Illinoian Glaciation of the Western Mesophytic Forest Region of Braun (1950); the Muscatatuck Flats and Canyons Section of the Bluegrass Natural Region of Homoya et al. (1985) and the *Fagus-Acer* presettlement vegetation category of Lindsey et al. (1965). The forest is relatively undisturbed with cutting of only dead trees, although grazing may have historically occurred in the stand (Jackson & Barnes 1975). Jackson and Barns (1975) found the woods to be dominated by American beech (*Fagus grandifolia* Ehrh.), sweet (red) gum (*Liquidambar styraciflua* L.) and red maple

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(*Acer rubrum* L.); vascular plant taxonomy follows Gleason and Cronquist (1991). The stand is characterized by low tree density and high basal area (Jackson & Barnes 1975) and a mean canopy closure of 76.6% (at tip up pools studied in this investigation). Tribbetts Woods is considered a "flatwoods" stand [or "pin oak land" or "crawfish land" (Chapman 1942)] due to the homogeneous, flat topography and poorly drained Clermont silt loam soils of the site. The soil of the forest is acidic with a mean pH of 5.0 (Jackson & Barnes 1975) and contains low organic matter (Chapman 1942). The soil exhibits extremely poor drainage (Braun 1950) and remains waterlogged through early summer (Chapman 1942; Jackson & Barnes 1975). Chapman (1942) provides a complete description of soil strata origin and formation.

Throughout Tribbetts Woods there is only a 0.6 m change in elevation (Jackson & Barnes 1975). Despite the homogeneity in elevation throughout the stand, microtopography changes are evident throughout the forest. Tip-up pit and mounds created by individual treefall events are very common in the flatwoods. When trees fall, the root wad and soil remain intact to form a large mound and a corresponding pit. Pits as wide as 4.75 m and as deep as 53 cm (Verb, personal observation) can be found throughout the stand (Figure 1). These pits maintain pools standing through mid-summer (Jackson & Banes 1975).

METHODS

In June 2009, nine tip-up pools from Tribbetts Woods were visited to determine their diatom composition. At each pool, a transect was established along the widest part of the pool. Along this transect five randomly selected sampling sites were established. At each sampling sites a portion of the surface sediments were extracted with a sterile pipette. All five sediment samples were combined as a composite sample and preserved with 2.5% $CaCO_3$-buffered glutaraldehyde. At each sampling site a leaf was collected off of benthos and scraped for periphyton using a stiff, sterile toothbrush. Scraped material from the five leaves at each sampling point were rinsed with distilled water, collected as a composite sample, and preserved with 2.5% $CaCO_3$-buffered glutaraldehyde in sterile 20-mL scintillation vials.

For each sample, a small (0.015 ml), homogenized quantity was placed in a Palmer-Maloney counting chamber to survey the basic shape morphologies and condition of diatoms (living or dead). A 10 ml periphyton subsample was extracted and cleaned using 30% H_2O_2 and concentrated HNO_3 (Stoermer et al. 1995). The cleaned samples were prepared on slides using NAPRAX^{TM} and 600–1000 valves were enumerated at 1000× using a Meiji MX4300L light microscope. Diatoms were identified to the species level when possible, using Patrick and Reimer (1966, 1975) and Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b).

RESULTS AND DISCUSSION

A total of 80 infrageneric diatom taxa were recorded from the tip-up pools (Table 1). The sediment samples yielded a total of 39 diatom taxa compared to 71 found in the leaf litter samples. Nine diatom taxa were exclusive to the pool sediments compared to 45 which were only found growing on the leaf litter. There were 26 taxa found utilizing both substrates (Table 1). In the epipelic samples diatoms with well-developed raphe systems (e.g., *Pinnularia*, *Frustulia*, *Nitzschia*) composed most of the species richness (29 taxa, 74%) while those diatoms of limited/no motility (e.g., *Meridion*) accounted for 26% (10 taxa) of the richness. Diatom communities found on the leaf litter showed a similar pattern with the more mobile taxa comprising 77% (55 taxa) of the species richness compared to 23% (15 taxa) for the diatoms of low mobility.

While diatoms are often mentioned as common and important constituents of temporary ponds (e.g., Masters, 1968), there are few studies that provide detailed surveys of the diatom species in ephemeral pools. Our total number of taxa reported (80) is comparable to Laird (1988) who reported (102–114 species) from three temporary pools in Ungava (Province of Quebec, Canada). The epipelic community in the tip-up pools was dominated by an abundance of highly motile and unattached diatoms displaying symmetrical biraphid (e.g., *Pinnularia*, *Frustulia*) or nitzschioid functional morphology (*sensu* Spaulding et al. 2010). In the sediment dominated habitats, the motile diatoms have a distinct advantage over their stalked, less motile, or immobile counterparts because they can utilize their well-developed raphes to avoid burial from settling particles

Figure 1.—Tip-up pool with the 4.5-m tall parental root mound in the background.

Table 1.—List of diatom taxa found inhabiting tip-up pools in Tribbetts Woods. Taxa with a "+"were found on the given substrates while those with "−" were not found.

Taxa	Sediments	Leaf litter
Achnanthes sp.	−	+
Achnanthidium minutissimum (Kütz.) Czarn.	+	+
Caloneis sp.	+	+
Caloneis bacillum (Grunow) Cleve	+	+
Caloneis cf *hyalina* Hust.	+	+
Caloneis cf *thermalis* (Grunow) Krammer	−	+
Cymbella sp.	−	+
Diadesmis aerophila (Krasske) D.G. Mann	−	+
Diadesmis contenta (Grun. *ex* Van Heurck) D.G. Mann	+	−
Diploneis sp.	−	+
Diploneis oblongella (Nägeli *ex* Kützing) Cleve-Euler	−	+
Encyonema mesianum (Cholnoky) D.G.Mann	−	+
Encyonema minutum (Hilse) D.G.Mann	−	+
Encyonema obscurum (Krasske) D.G. Mann	−	+
Encyonema silesiacum (Bleisch *ex* Rabenh.) D.G. Mann	−	+
Eunotia spp.	+	+
Eunotia sp. #1	−	+
Eunotia arcus Ehrenb.	−	+
Eunotia bilunaris (Ehrenb.) Mills	−	+
Eunotia curvata (Kütz.) Lagerst.	+	+
Eunotia exigua (Bréb.) Rabenh.	+	+
Eunotia exigua (Bréb.) Rabenh. - 3 lobed	−	+
Eunotia implicata Nöepel, Lange-Bert. & Alles	−	+
Eunotia cf *incisa* W.Sm.	−	+
Eunotia cf *minor* (Kütz.) Grunow	−	+
Eunotia praerupta Ehrenb.	−	+
Eunotia cf *subarcuatoides* Alles, Nörpel & Lange-Bertalot	+	+
Eunotia cf *tenella* (Grunow) Hust.	+	−
Frustulia rhomboides (Ehrenb.) DeToni	+	+
Frustulia vulgaris (Thwaites) DeToni	−	+
Gomphoneis olivacea (Hornemann) P.A.Dawson *ex* R.Ross & P.A.Sims	−	+
Gomphonema spp.	+	+
Gomphonema angustatum (Kütz.) Rabenh.	−	+
Gomphonema minutum (C.Agardh.) C.Agardh.	−	+
Gomphonema parvulum (Kütz.) Kütz.	+	+
Hantzschia amphioxys (Ehrenb.) Grunow	−	+
Luticola saxophila (Bock *ex* Hustedt) D.G.Mann	−	+
Melosira sp.	+	−
Meridion circulare (Grev.) C.Agardh	−	+
Navicula spp.	+	−
Navicula sp. #1	−	−
Navicula cf *digitulus* Hust.	−	+
Navicula minima Grunow	−	+
Navicula minuscula Grunow	−	+
Neidium sp. #1	−	+
Neidium affine (Ehrenb.) Pfitz.	+	+
Neidium ampliatum (Ehrenb.) Krammer	+	+
Neidium binodeforme Krammer	−	+
Neidium dubium (Ehrenb.) Cleve	−	+
Neidium productum (W.Smith) Cleve	−	+
Nitzschia spp.	+	+
Nitzschia capitellata Hust.	+	+
Nitzschia clausii Hantzsch	−	+
Nitzschia dissipata (Kütz.) Grunow	+	+

Table 1.—Continued.

Taxa	Sediments	Leaf litter
Nitzschia frustulum (Kütz.) Grunow	−	+
Nitzschia inconspicua Grunow	+	−
Nitzschia microcephala Grunow	−	+
Nitzschia palea (Kütz.) W.Sm.	+	+
Nitzschia perminuta (Grunow) M.Perag.	−	+
Nitzschia recta Hantzsch	−	+
Pinnularia spp. (girdle view)	+	+
Pinnularia sp. #1	−	+
Pinnularia acoricola Hustedt	+	−
Pinnularia cf *acrospaeria* Raben.	−	+
Pinnularia brauniana (Grunow) Mills	+	+
Pinnularia divergentissima (Grunow) Cleve	+	+
Pinnularia gibba Ehrenb.	+	+
Pinnularia intermedia (Lagest.) Cleve	+	−
Pinnularia nodosa (Ehrenb.) W.Sm.	−	+
Pinnularia obscura Krasske	+	+
Pinnularia microstauron (Ehrenb.) Cleve	+	+
Pinnularia subcapitata W.Greg.	+	+
Pinnularia viridis (Nitzsch) Ehrenb.	+	+
Planothidium lanceolatum (Bréb. *ex* Kützing) Lange-Bert.	−	+
Reimeria sinuata (W. Greg.) Kociolek & Stoermer	+	−
Stauroneis anceps Ehrenb.	−	+
Stauroneis kriegeri R.M.Patrick	−	+
Stauroneis phoenicenteron (Nitzsch) Ehrenb.	+	+
Stauroneis producta Grunow	−	+
Stauroneis smithii Grunow	+	−

(Stevenson & Bahls 1999). The abundance of *Pinnularia* in the tip-up pools correlates with other studies that have noted its dominance in ephemeral pond systems (Williams et al. 2004).

With almost twice as many diatom species as the epipelic habitat, the leaf litter seemed to be particularly influential on diatom richness in the tip-up pools. In vernal pools leaf litter has been noted to serve as solid substrate for colonization by microorganisms (Williams 2006). The sturdy foundational nature allowed diatoms with slight motility (e.g., *Eunotia*) and those able to produce stalks (e.g., *Gomphonema*) to form a firm attachment point. Furthermore, the species richness on the leaf litter was also enhanced by the presence of a fine layer of sediment on the surface. This covering allowed for many mobile taxa to inhabit the leaf litter, thus creating a mixed diatom habitat, which perhaps in part contributed to the greater number of species in these samples when compared to the sediments alone. This sediment deposition on the leaves is most likely due to repeated turbidation and the resettling of the sediments, along with additional erosional contributions from the root wads as the soil erodes back into the pool (Rubino, personal observation). The abundance of *Eunotia* taxa, specifically *E. exigua*, on the leaf litter was unusual since they are successful in waters with a pH$<$ 5.5 (Lowe 1974; Van Damm *et al.*, 1994; Furey 2010) and the water column of these pools ranged from 5.54–6.20 (Verb, unpublished data). While the water column was not overly acidic, perhaps the accumulation/release of organic acids due to microbial conditioning and decomposition formed a suitable microhabitat for the acidophilic diatoms (Friberg & Winterbourn 1996; Colburn 2004) and/or influence of the acidic soil (Jackson & Barnes 1975).

In conclusion, this report describes a mere snapshot of the diatom communities in these tip-up pools of Illinoian tillplain forests in southeastern Indiana. It confirms our suspicions that these pools serve as a viable refugia and habitat within the forest system for diatoms. This survey can serve as a foundation from which additional studies can investigate the impact of environmental variables and interactions between different organisms in these unique temporary systems.

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